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Integrated *In vivo* and *In Silico* Evaluation of Phenolic-Rich Extract of *Euphorbia hirta* Against Inflammatory Bowel Disease

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KEYWORDS
Euphorbia hirta, Inflammatory Bowel Disease (IBD), Dextran Sodium Sulfate (DSS), Anti-inflammatory activity, Cytokines

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ABSTRACT
Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract characterized by oxidative stress, immune dysregulation and mucosal damage. The limitations of current therapies have increased interest in plant-based alternatives with anti-inflammatory potential. The present study evaluated the anti-inflammatory activity of a phenolic-rich extract of *Euphorbia hirta* through integrated phytochemical, in-vivo and in-silico approaches. The plant material was collected, authenticated and processed to obtain a phenolic-rich extract. Phytochemical profiling using Gas Chromatography–Mass Spectrometry (GC–MS) and High-Performance Liquid Chromatography (HPLC) confirmed the presence of major phenolic compounds, including rutin and quercetin. The anti-inflammatory activity was investigated in a dextran sodium sulfate (DSS)-induced experimental model of inflammatory bowel disease in Wistar rats. Animals were divided into five groups (n = 6 per group): control, DSS-induced disease control, standard, and treatment groups. The phenolic-rich extract was administered orally at a dose of 250 mg/kg and 500mg/kg body weight, while the standard group received metronidazole (100mg/kg, p.o.). Disease activity index, body weight changes, histopathological alterations and inflammatory responses were assessed to evaluate the therapeutic efficacy of the extract. Treatment with the extract demonstrated significant protective effects against intestinal inflammation and tissue damage compared with the disease control group ($p < 0.05$). Molecular docking studies were performed to examine the interactions of rutin and quercetin with inflammation-related protein targets. Both compounds exhibited favourable binding affinities, supporting their potential role in modulating inflammatory pathways. The findings suggest that *Euphorbia hirta* possesses significant anti-inflammatory activity and may serve as a promising natural therapeutic candidate for the management of IBD, supporting its traditional medicinal use and future therapeutic applications.

INTRODUCTION

The present study is designed to evaluate the protective effect of a phenolic-rich extract of *Euphorbia hirta* against inflammatory bowel disease using DSS-induced experimental colitis in animal models. The study also aims to characterize the extract using UV-Vis, FTIR and HPLC techniques and to examine interactions of major phytoconstituents with inflammatory targets through in silico molecular docking. This integrated approach may provide scientific evidence for the development of effective, affordable and plant-based therapies for IBD. The present study was undertaken to evaluate the phytochemical composition and therapeutic potential of the ethanolic extract of *Euphorbia hirta* against DSS-induced inflammatory bowel disease using integrated phytochemical, in vitro, in vivo and in silico approaches. The findings obtained from the study demonstrate that the extract possesses significant antioxidant and anti-inflammatory activities, which may be attributed to its rich phenolic and flavonoid content.

The physicochemical standardization studies, including ash values, loss on drying and extractive values, confirmed the quality and purity of the plant material. The higher alcohol-soluble extractive value and extraction yield obtained with ethanol suggest that ethanol was an efficient solvent for extracting bioactive phytoconstituents from *Euphorbia hirta*. Preliminary phytochemical screening further revealed the presence of flavonoids, phenolic compounds, alkaloids, glycosides and amino acids, which are known to contribute to various pharmacological activities. These observations are

consistent with previous reports indicating that *Euphorbia hirta* is rich in biologically active secondary metabolites. 14,15

Fig.1: *Euphorbia hirta*

MATERIAL AND METHODS

MATERIALS

Plant Material

The whole plant of *Euphorbia hirta* was collected from Tirumala Hills, Chittoor District, Andhra Pradesh, India. The collected plant material was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical or HPLC grade. Ethanol, methanol, acetonitrile and formic acid were used for extraction and chromatographic analysis. Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, sodium nitrite and sodium hydroxide were used for phytochemical estimations. Antioxidant assays were carried out using reagents such as DPPH, sodium nitroprusside, Griess reagent, hydrogen peroxide, reduced glutathione (GSH), EDTA and trichloroacetic acid (TCA). Dextran sulphate sodium (DSS) was used for the induction of colitis. Other chemicals such as potassium dichromate, acetic acid, phosphate buffered saline (PBS), hydrochloric acid and distilled water were also used throughout the study.

Standards

Rutin and quercetin were used as reference standards for the estimation of total phenolic content, total flavonoid content and HPLC analysis of the extract. Metronidazole was used as the standard drug for the in vivo study.

Instruments and Equipment

The study was carried out using standard laboratory instruments including a Soxhlet extraction apparatus, UV–Visible spectrophotometer, ATR-FTIR spectrophotometer, HPLC system equipped with an XBridge C18 column and GC–MS system (PerkinElmer Clarus 500). In addition, basic laboratory equipment such as an analytical balance, hot air oven, muffle furnace, centrifuge, water bath, pH meter, micropipettes and desiccator was used during the experimental work.

Experimental Animals

Healthy Wistar rats weighing 150–200g were used for the in vivo anti-inflammatory study. The animals were maintained under standard laboratory conditions with a controlled environment and they were given free access to standard pellet diet and water throughout the experimental period. All animal experimental procedures were performed in accordance with standard ethical guidelines.

METHODOLOGY

Collection and identification of plant material

The plant *Euphorbia hirta* was collected from Tirumala hills, Tirumala, Chittor Dt, AP, India. It was identified and validated by a government recognized botanist Dr. K. Madhava Chetty, Assistant Professor, Department of botany, Sri Venkateswara University, Tirupati, A.P, India.

Standardization of powdered drug (Khandelwal, K.R. 1999[24] & WHO, 2002– 2005[25])

Determination of ash values

The quality and purity of a crude drug were determined by ash values. Ash is an inorganic remnant of a crude drug resulted after its incineration at a temperature of 450˚C in muffle furnace. Ash value gives a measure of total amount of minerals within a sample. The ash remained after ignition of crude drug was measured for its total ash content, acid insoluble ash content and water insoluble ash content by three different methods.

a) Total ash value

The total ash value evaluates the total amount of powered crude drug remained after detonation at a temperature of 450˚C to remove all the carbon atoms. It includes the plant tissue derived physiological ash and the plant surface adhered extraneous matter residue non physiological ash. A high ash value indicates a greater number of impurities in the crude drug.

Procedure

Powdered crude drug was weighed and heated in a crucible till the vapors completely ceases. The obtained ash was deprecated at high temperature of 450˚C till it appears white, signifying the lack of carbon. Ash was subsequently cooled in desiccator, weighed and then moistened initially with hot water in a pre-cooled crucible. The residue collected in ash less filter paper was cauterized at a temperature not surpassing 450˚C. Then it was moistened again with alcohol the above procedure was followed similarly. In a suitable desiccator, for a period of 30 min the residue was cooled and then weighed immediately. The total ash content was calculated on a percentage basis with reference to the air-dried sample of the powdered crude drug.

b) Acid insoluble ash value

Procedure

The obtained total ash was poached for 5 minutes with 2M hydrochloric acid and insoluble inorganic matter was poised in ash free filter paper. The filter paper with insoluble matter is heated gently in a crucible until vapors ceases to be evolved. The obtained was kept for incineration at 450˚C temperature. Let the residue to cool for 30 minutes in a suitable desiccator and taken its weight. The acid insoluble ash content was estimated on a percentage basis with reference to the air-dried sample of the powdered crude drug.

c) Water soluble ash value

Water soluble ash content is the part of the total ash content that soluble in water.

Procedure

The total ash content was weighed and poached with 25 ml of water for a period of 5 minutes. Ash less filter paper with the collected insoluble matter was washed with warm water and cauterized at a temperature not greater than 450˚C.The difference in weight obtained by subtracting the weight of insoluble matter from the weight of ash is represented as water

soluble ash. The water insoluble ash content was calculated on a percentage basis with reference to the air-dried sample of the powdered crude drug.

Loss on drying

Loss on drying or moisture content is the loss of mass expressed as percentage w/w. In a crude drug the water and volatile matter was determined by the test for loss on drying. The drying was performed by heating the sample at 100-105˚C.

A) Powdered leaves of Euphorbia hitra used for extraction
B) Before Extraction

C) After Extraction *D) Ethanolic Extraction*

Fig.2

Procedure

2-5grams of powered drug was taken in a porcelain dish and heated in hot air oven at about 100-105˚C for 5 hours. Drying under a constant weight, the sample was permitted to cool in a desiccator before weighing. Drying process was continued to an extent that two successive weights should not alter greater by 5mg. The weight loss was estimated in terms of mg per gram of crude drug material by weighing the porcelain dish and its contents.

Extractive value

Extractive value discloses the amount of chemical constituents of a plant material when extracted with different solvents. The extractive amount to a given solvent is a measure of a particular type of constituents or a group of similar constituents contained in the sample. The extractive solvent should be efficient to dissolve considerable quantities of substances desired.

a) Alcohol soluble extractive value

20grams of powdered leaf of *Euphorbia hirta* was macerated with 100ml of alcohol for 24 hours with frequent shaking for first 8 hours and was permitted to stand for the next 18 hours. The macerate was filtered quickly and precaution taken to prevent loss of alcohol. Filtrate of 25ml was then evaporated to dryness at 105˚C and then weighed. The percentage of alcohol soluble extract had been determined with reference to the air-dried drug.

b) Water soluble extractive value

The procedure performed for estimation of water-soluble extractive value is followed similarly for alcohol soluble extractive evaluation; except for the solvent used was chloroform-water instead of ethanol.

c) Ether soluble extractive value

Roughly powdered drug of 100gm was taken and extracted with solvent ether in a continuous extraction apparatus for 6 hours. The extract obtained was filtered and 10ml of it was transferred to a tarred evaporating dish. The residue resulted after solvent evaporation was dried on water bath at 105˚C to constant weight. The percentage-based ether soluble extractive value was estimated with referral to the air-dried drug.

Preparation of extract

After collection of the plant, shade dried leaves of *Euphorbia hirta* were then blended in to fine powder with a blender and used for the ethanol extract. Ethanol extract was extracted by using Soxhlet extractor for 18-20 h. The extract obtained, was concentrated under reduced pressure at controlled temperature (40-50˚C) and finally made powdered[26].

In Vitro Antioxidant Activity

Free radicals may be defined as any chemical species that are capable of existing with one or more unpaired outer shell electrons. They are extremely reactive and generally highly unstable. Reactive oxygen species, such as superoxide radical, hydrogen peroxide (H2O2), hydroxyl radical (OH_) and singlet oxygen (1 O2), are of the greatest biological significance. The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources. Synthetic Antioxidant cause toxicity therefore recently there has been an upsurge of interest in therapeutic potential of medicinal plants as antioxidants in reducing such free radicals.

Free Radical Scavenging Activity on DPPH reagents

DPPH reagent, Methanol

Procedure

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois (1958). The sample extracts at various concentrations (50 - 250µg) was taken and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1mM methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Percentage radical scavenging activity of the sample was calculated as follows:

% DPPH radical scavenging activity = (control OD-sample OD / control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC50) under the assay condition was calculated from the graph of inhibition percentage against sample concentration[34]

RESULTS AND DISCUSSION

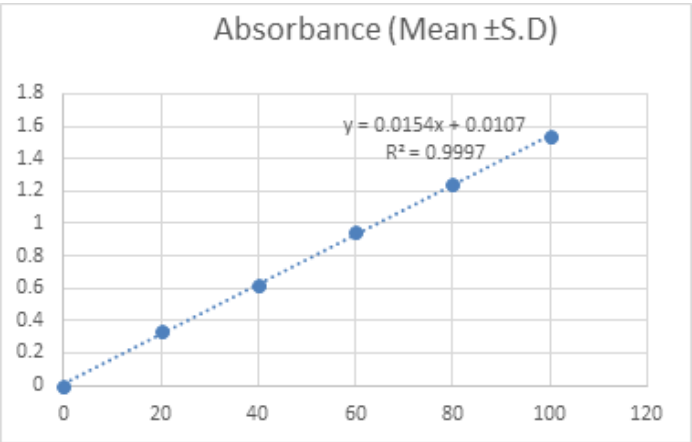


Figure.3: Standard Calibration Curve of Quercetin for Total Phenolic Content Estimation

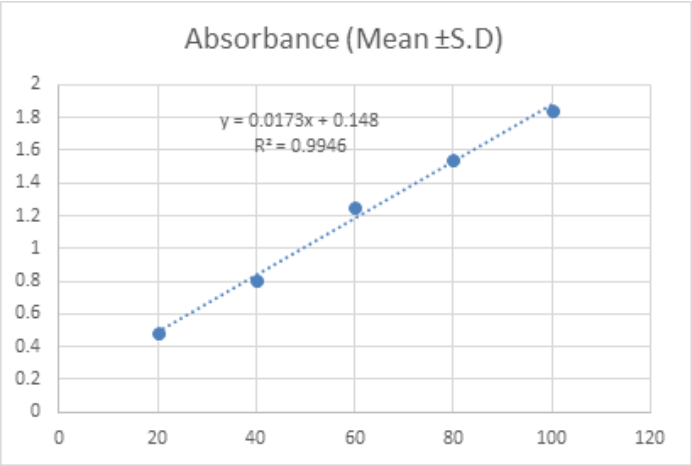


Figure.4: Standard Calibration Curve of Quercetin for Total Flavonoid Content Estimation

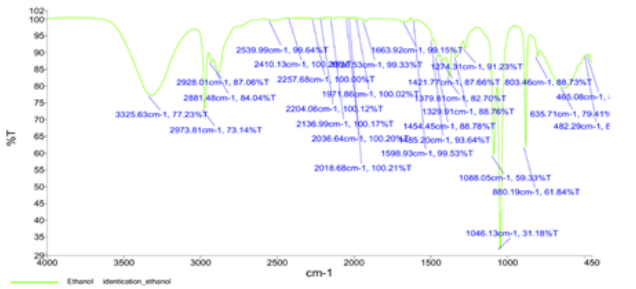


Figure.5: FTIR spectrum of ethanolic extract of *Euphorbia hirta* showing characteristic absorption peaks

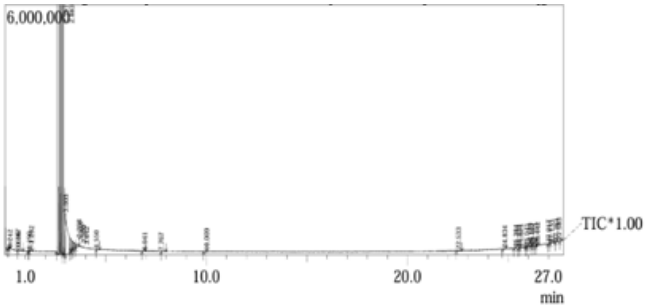
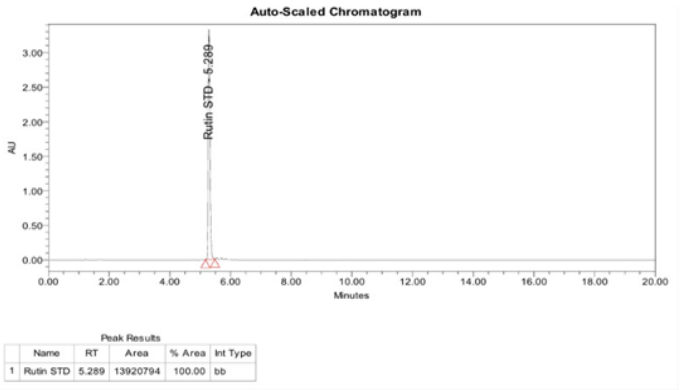


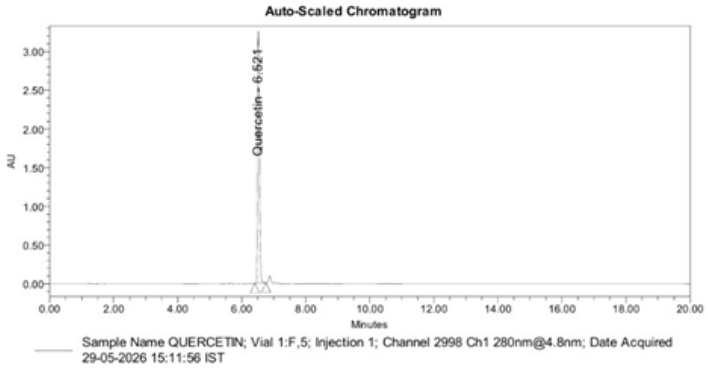
Figure.6: GC-MS Chromatogram of *Euphorbia hirta* Extract



Peak Results

	Name	RT	Area	% Area	Int Type
1	Rutin STD	5.289	13920794	100.00	bb

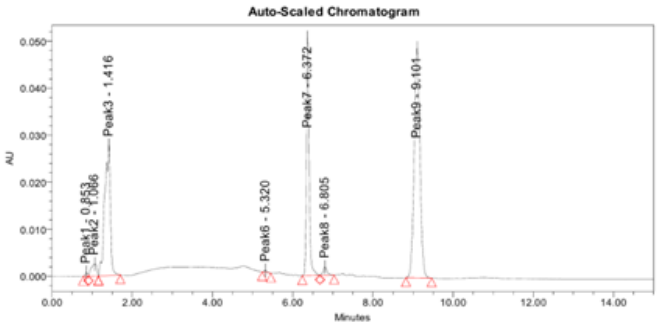
Figure.7: RP-HPLC chromatogram of rutin standard showing a retention time of 5.289 min


Auto-Scaled Chromatogram
Quercetin - 6.521
AU
Minutes
Sample Name QUERCETIN; Vial 1:F,5; Injection 1; Channel 2998 Ch1 280nm@4.8nm; Date Acquired 29-05-2026 15:11:56 IST

Peak Results

	Name	RT	Area	% Area	USP Plate Count	USP Tailing	USP Resolution
1	Quercetin	6.521	12534162	100.00	72172	1.2	

Figure.8: RP-HPLC chromatogram of quercetin standard showing a retention time of 6.521 min



Peak Results

	Name	RT	Area	% Area	Int Type
1	Peak1	0.853	3323	0.32	BV
2	Peak2	1.066	26111	2.51	VB
3	Peak3	1.416	271443	26.06	BB
4	Peak4	1.468			Missing
5	Peak5	4.792			Missing
6	Peak6	5.320	2289	0.22	BB
7	Peak7	6.372	236497	22.70	BV
8	Peak8	6.805	11656	1.12	VB
9	Peak9	9.101	490350	47.07	BB

Figure.9: RP-HPLC chromatogram of ethanolic extract of *Euphorbia hirta* showing peaks corresponding to rutin, quercetin and other phytochemical constituents.

In-Vitro Antioxidant and Free Radical Scavenging Activity

The reactive oxygen species play an important role related to the degenerative or pathological processes of various serious diseases such as ageing, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation. Hence, the agents that can scavenge these reactive oxygen species can be

beneficial in the treatment of inflammatory disorders. The antioxidant activity of ethanolic extract of *Euphorbia hirta* were done for DPPH radical scavenging activity, and nitric oxide radical scavenging activity using standard procedures.

DPPH Free Radical Scavenging Activity

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract. Scavenging of DPPH radical is related to the inhibition of lipid peroxidation. DPPH is usually used as a substance to evaluate the antioxidant activity. Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radicals. The molecule 1, 1-diphenyl-2- picrylhydrazyl (a,a-diphenyl-bpicrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals.

The delocalization of electron also gives rise to the deep violet color. The DPPH radical scavenging activity of Ethanolic extract of *Euphorbia hirta* were detected and compared with Ascorbic acid from 50-250 µg/ml. The percentage of inhibition was increased from 7.19 % to 51.12 % The IC50 value of Ethanolic extract of *Euphorbia hirta* was 39.99± 0.05. The inhibitory percentage of Ethanolic extract of *Euphorbia hirta* was increased with increased concentration of extract in Dose-dependent manner.

Ethanolic Extract of *Euphorbia hirta* showed a concentration-dependent increase in antioxidant potential. The percentage inhibition increased gradually from 7.19±0.11% at 50µg/ml to 51.12±0.22% at 250µg/ml. The IC_{50} value of the ethanolic extract was found to be 39.99±0.05 µg/ml, indicating significant antioxidant activity. The results suggest that the ethanolic extract of ***Euphorbia hirta*** possesses appreciable free radical scavenging potential, which may be attributed to the presence of phytoconstituents such as phenolic compounds and flavonoids. The antioxidant activity increased with increase in concentration of the extract, demonstrating dose-dependent radical scavenging activity.

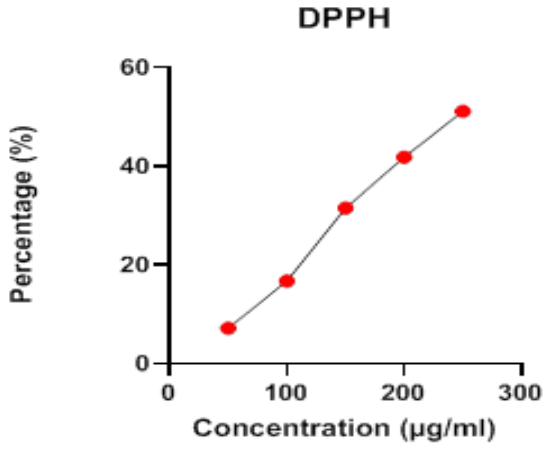


Figure.10: DPPH free radical scavenging activity of ethanolic extract of *Euphorbia hirta* at different concentrations

Nitric Oxide Radical Scavenging Activity

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases. It would be interesting to develop potent and selective inhibitors of NO for potential therapeutic use. Nitric oxide radical scavenging activity of Ethanolic Leaf extract of *Euphorbia hirta* were detected and compared with the standard ascorbic acid. The percentage inhibition (% inhibition) at various concentration (50- 250 µg/ml) of as well as standard Ascorbic acid were calculated and plotted in (Table 4.13 & Figure 1.11). The IC50 values are calculated from graph and were found Ascorbic acid (100.71 ± 3.98 µg/ml) and the IC50 values of Ethanolic extract of *Euphorbia hirta* is 10.32 ± 0.03µg/ml).

The nitric oxide radical scavenging activity of the ethanolic extract of Euphorbia hirta showed a concentration-dependent increase in percentage inhibition. The percentage inhibition increased gradually from 7.26 ± 0.73% at 50µg/ml to 50.20 ± 1.45% at 250µg/ml. The IC_{50} value of the ethanolic extract was found to be 10.32 ± 0.03 µg/ml, indicating significant nitric oxide radical scavenging activity. The results suggest that the ethanolic extract of *Euphorbia hirta* possesses appreciable antioxidant potential and may contribute to neutralization of nitric oxide radicals due to the presence of bioactive phytoconstituents.

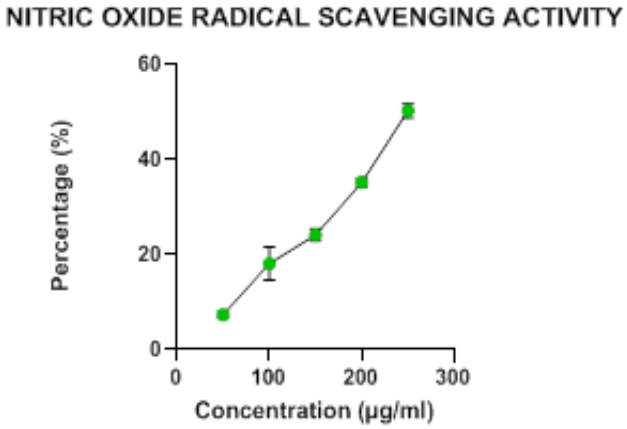


Figure.11: Nitric oxide radical scavenging activity of ethanolic extract of Euphorbia hirta at different concentrations

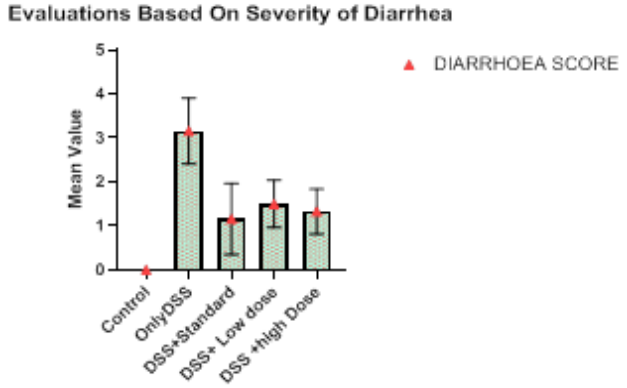


Figure.12: Evaluation of antidiarrheal activity based on severity of diarrhoea in experimental groups

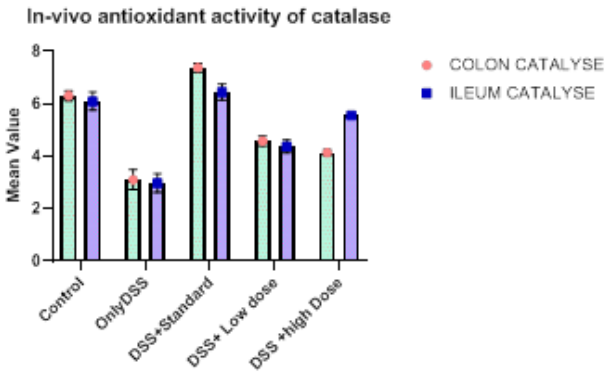


Figure.13: Effect of ethanolic extract of Euphorbia hirta on catalase activity in colon and ileum tissues of DSS-induced inflammatory bowel disease rats

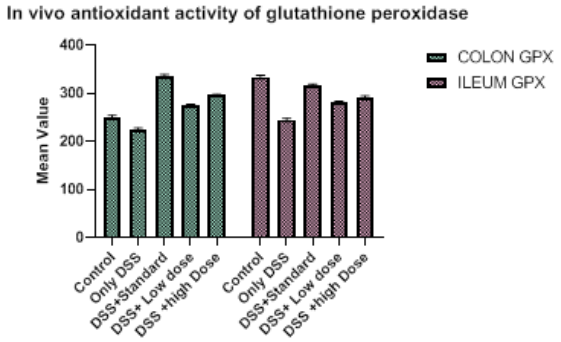


Figure.14: Glutathione peroxidase activity in colon and ileum tissues of different experimental groups

Molecular docking: Molecular docking studies were performed to evaluate the binding affinity of the major phytoconstituents, Rutin and Quercetin, against selected inflammatory targets associated with Inflammatory Bowel Disease (IBD). The target proteins selected for the study were SRC kinase (1Y57), Matrix Metalloproteinase-9 (MMP9; 1L6J), Kinase Insert Domain Receptor (KDR; 3VHE), AKT1 (4EJN), Interleukin-6 (IL-6; 1ALU), Nuclear Factor-kappa B (NF-κB; 1NFI), Tumor Necrosis Factor-alpha (TNF-α; 2AZ5), Inducible Nitric Oxide Synthase (iNOS; 3E7G) and Cyclooxygenase-2 (COX-2; 5IKR). The docking analysis was carried out using Auto Dock Vina and the best binding affinities were recorded.

Molecular Docking Scores

Table 1: Binding affinity of Rutin and Quercetin against selected target protein

Protein	PDB ID	Ligand	Binding Affinity (kcal/mol)
SRC	1Y57	Rutin	-7.1
SRC	1Y57	Quercetin	-7.6
MMP-9	1L6J	Rutin	-8.7
MMP-9	1L6J	Quercetin	-9.9
KDR	3VHE	Rutin	-0.0
KDR	3VHE	Quercetin	-0.0
AKT-1	4EJN	Rutin	-11.4
AKT-1	4EJN	Quercetin	-9.4
IL-6	1ALU	Rutin	-4.7
IL-6	1ALU	Quercetin	-4.9
NF-κB	1NFI	Rutin	-8.0
NF-κB	1NFI	Quercetin	-6.5
TNF-α	2AZ5	Rutin	-0.0
TNF-α	2AZ5	Quercetin	-5.8
iNOS	3E7G	Rutin	-12.3
iNOS	3E7G	Quercetin	-10.3
COX-2	5IKR	Rutin	-8.2
COX-2	5IKR	Quercetin	-8.4

Protein–Ligand Interaction Analysis

The two-dimensional interaction analysis demonstrated the formation of hydrogen bonds and hydrophobic interactions between the ligands and amino acid residues present within the active sites of the target proteins. Among the analyzed complexes, rutin exhibited strong interactions with AKT1 and iNOS, whereas quercetin showed significant interactions with MMP-9 and iNOS. These interactions contributed to the stability of the protein–ligand complexes and were reflected by their favorable docking scores.

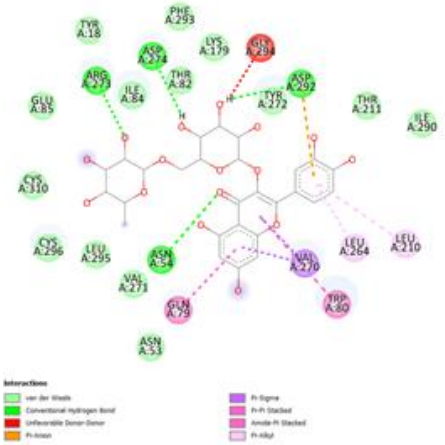


Figure.15: Interaction between ligand and receptor

Molecular Docking Results

The interaction residue analysis identified several amino acid residues involved in ligand binding within the active site regions of the target proteins. The AKT1–Rutin complex exhibited interactions with ASP274, ARG273, ASP292 and ASN54 residues. The AKT1–Quercetin complex showed interaction with ASP292 residue. In the MMP9–Quercetin complex, interactions were observed with MET422, PRO421 and ARG424 residues. The iNOS–Rutin complex demonstrated interactions with GLU377 and TRP372 residues, whereas the iNOS–Quercetin complex showed interaction with TRP372 residue. These interactions were visualized through the 2D interaction diagrams and corresponding 3D binding pose analyses.

DISCUSSION

The total phenolic and total flavonoid content analysis demonstrated that the extract contained appreciable amounts of polyphenolic compounds. Phenolics and flavonoids are well recognized for their ability to neutralize free radicals and regulate inflammatory pathways, as reported in earlier studies 57–61. The presence of these compounds may therefore contribute significantly to the biological activities observed in the present investigation. FTIR analysis confirmed the presence

of important functional groups such as hydroxyl, carbonyl and aromatic groups, which are commonly associated with phenolic and flavonoid compounds. Similarly, GC-MS analysis identified several phytochemical constituents with reported antioxidant and anti-inflammatory properties, indicating the chemical diversity of the extract. HPLC analysis further confirmed the presence of important flavonoid markers, particularly rutin and quercetin, which have been extensively reported for their protective effects against oxidative stress and inflammation.

The in vitro antioxidant studies demonstrated that the ethanolic extract exhibited concentration-dependent free radical scavenging activity against both DPPH and nitric oxide radicals. These findings suggest that the extract possesses effective antioxidant potential capable of reducing oxidative stress. Since excessive generation of reactive oxygen species plays a major role in the pathogenesis of inflammatory bowel disease, the antioxidant activity of the extract may contribute to its protective effects against intestinal inflammation.

DSS-induced colitis is a well-established experimental model that closely resembles several clinical and pathological features of human ulcerative colitis, as previously reported1–3,17,37,38. Administration of DSS produced severe intestinal inflammation characterized by diarrhea, increased disease activity index, oxidative stress and tissue damage. Treatment with the ethanolic extract of *Euphorbia hirta* significantly improved these pathological changes in a dose-dependent manner, indicating its therapeutic potential against inflammatory bowel disease.

Oxidative stress is considered one of the major mechanisms involved in DSS-induced intestinal injury. In the present study, DSS administration significantly reduced the levels of endogenous antioxidant enzymes such as catalase, glutathione peroxidase and reduced glutathione in colon and ileum tissues. However, treatment with *Euphorbia hirta* extract restored these antioxidant parameters toward normal values.

The higher dose (500 mg/kg) produced greater improvement than the lower dose (250 mg/kg), suggesting dose-dependent antioxidant activity. These findings indicate that the extract effectively enhances the endogenous antioxidant defense system and protects intestinal tissues from oxidative damage. Myeloperoxidase activity is a widely accepted marker of neutrophil infiltration and intestinal inflammation. DSS treatment resulted in a marked increase in MPO activity, reflecting severe inflammatory responses within the colon. Administration of the extract significantly reduced MPO levels in a dose-dependent manner, demonstrating its anti-inflammatory effect. The reduction in MPO activity suggests suppression of inflammatory cell infiltration and attenuation of intestinal inflammatory responses.

Histopathological evaluation provided further evidence supporting the protective effects of the extract. Severe epithelial erosion, inflammatory cell infiltration, crypt distortion and mucosal damage were observed in the DSS-induced group. In contrast, animals treated with *Euphorbia hirta* showed marked improvement in tissue architecture, reduced inflammatory lesions and restoration of normal mucosal structure. The protective effect was more pronounced at the higher dose, indicating better preservation of intestinal integrity.

Network pharmacology analysis suggested that rutin and quercetin interact with multiple targets associated with inflammatory bowel disease and are involved in important signaling pathways such as PI3K-Akt, MAPK, TNF and NF-κB pathways. These pathways play critical roles in regulating inflammatory responses, oxidative stress and immune functions. Molecular docking studies further supported these observations by demonstrating favorable binding interactions of rutin and quercetin with several inflammation-related targets, including AKT1, MMP9 and iNOS. The strong binding affinities observed indicate that these compounds may contribute significantly to the anti-inflammatory activity of *Euphorbia hirta* through modulation of multiple molecular targets.

Overall, the findings of the present study demonstrate that the ethanolic extract of *Euphorbia hirta* possesses significant antioxidant and anti-inflammatory activities. The protective effects observed against DSS-induced colitis may be attributed to the synergistic action of phenolic compounds, particularly rutin and quercetin, which help reduce oxidative stress, suppress inflammatory responses and preserve intestinal tissue integrity. The integrated phytochemical, biological and computational evidence obtained in this study provides strong scientific support for the traditional medicinal use of *Euphorbia hirta* and highlights its potential as a promising natural therapeutic agent for the management of inflammatory bowel disease.

CONCLUSION

The present study was conducted to evaluate the protective potential of a phenolic-rich ethanolic extract of *Euphorbia hirta* against DSS-induced inflammatory bowel disease using a combination of phytochemical, antioxidant, in vivo, network pharmacology and molecular docking approaches.

The results obtained from the study demonstrated that *Euphorbia hirta* possesses considerable antioxidant and anti-inflammatory activities, which may contribute to its beneficial effects in inflammatory bowel disease. Pharmacognostic and phytochemical investigations confirmed the quality of the plant material and revealed the presence of several important secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, glycosides, steroids and triterpenoids. Quantitative analysis showed that the extract was rich in phenolic and flavonoid compounds, indicating strong antioxidant potential. Furthermore, FTIR, GC–MS and HPLC analyses confirmed the presence of various bioactive constituents, particularly rutin and quercetin, which are known for their significant antioxidant and anti-inflammatory properties. The extract exhibited notable free radical scavenging activity in both DPPH and nitric oxide assays, demonstrating its ability to neutralize reactive oxygen and nitrogen species. Since oxidative stress is an important factor in the progression of inflammatory bowel disease, the observed antioxidant activity may play a crucial role in protecting intestinal tissues from oxidative damage.

In the DSS-induced colitis model, treatment with *Euphorbia hirta* effectively reduced disease severity and improved clinical

manifestations associated with intestinal inflammation. The extract significantly restored endogenous antioxidant enzymes, including catalase, glutathione peroxidase and reduced glutathione, while decreasing myeloperoxidase activity. These findings indicate its ability to enhance antioxidant defense mechanisms and suppress inflammatory responses. Histopathological examination further supported these observations by demonstrating improvement in colonic tissue architecture, reduction of inflammatory cell infiltration and preservation of mucosal integrity in treated animals.

Network pharmacology analysis suggested that the major phytoconstituents, rutin and quercetin, interact with multiple targets involved in inflammatory bowel disease. These targets were associated with important signaling pathways such as PI3K-Akt, MAPK, TNF and NF-κB, which play key roles in inflammation, immune regulation and oxidative stress. This indicates that the therapeutic effects of Euphorbia hirta are likely mediated through multiple molecular targets and signaling pathways.

Molecular docking studies further strengthened these findings by demonstrating favorable binding interactions of rutin and quercetin with several important inflammatory targets, including SRC kinase, AKT1, MMP9, KDR, IL-6, TNF-α, NF-κB, iNOS and COX-2. The strong binding affinities observed suggest that these compounds may contribute significantly to the modulation of inflammatory pathways involved in disease progression.

Overall, the findings of the present investigation demonstrate that the phenolic-rich ethanolic extract of *Euphorbia hirta* possesses significant antioxidant and anti-inflammatory activities and provides protection against DSS-induced intestinal inflammation. The beneficial effects observed in the study may be attributed to the combined action of its bioactive constituents, particularly rutin and quercetin, through the regulation of oxidative stress and inflammatory mediators. The integration of phytochemical characterization, biological evaluation, network pharmacology and molecular docking studies provides strong scientific support for the therapeutic potential of *Euphorbia hirta*. In conclusion, the present study provides scientific evidence supporting the traditional use of *Euphorbia hirta* and highlights its potential as a promising natural therapeutic candidate for the management of inflammatory bowel disease. Further investigations involving isolation of active constituents, detailed mechanistic studies, long-term safety assessment and clinical evaluation are required to facilitate its future development as a safe and effective phytopharmaceutical agent.

FUTURE SCOPE

- Long-term toxicity and safety evaluations to establish its therapeutic suitability.
- Assessment of purified compounds in advanced experimental models of inflammatory bowel disease.
- Clinical investigations to validate the safety and efficacy of *Euphorbia hirta* in human subjects.
- Development of standardized herbal formulations and phytopharmaceutical products based on its active constituents.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

1837/PO/RcBiBt/S/2015/CCSEA

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

[1] Danese S, Fiocchi C. Ulcerative colitis and Crohn's disease. World J Gastroenterol. 2011;17(1):1–10.
[2] Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017; 389: 1756–1770.
[3] Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: A systematic review of population-based studies. Lancet. 2017; 390: 2769–2778.
[4] Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007; 448: 427–434.
[5] Baumgart DC, Sandborn WJ. Inflammatory bowel disease: Clinical aspects and established therapies. Lancet. 2012; 380:1590–1605.
[6] Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol. 2014; 14: 329–342.
[7] Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 2009;9:799–809.
[8] Atreya I, Atreya R, Neurath MF. NF-κB in inflammatory bowel disease. J Intern Med. 2008; 263: 591–596.
[9] Rezaie A, Parker RD, Abdollahi M. Oxidative stress in inflammatory bowel disease: An overview. Dig Dis Sci. 2007; 52: 2015–2021.
[10] Hanauer SB. Medical therapy for ulcerative colitis. Gastroenterology. 2004; 126: 1582–1592.
[11] Lichtenstein GR, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Price S, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin Gastroenterol Hepatol. 2012; 10:462–469.
[12] Calixto JB. The role of natural products in modern drug discovery. An Acad Bras Cienc. 2019; 91: e20190105.
[13] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009; 2: 270–278.
[14] Kumar S, Malhotra R, Kumar D. *Euphorbia hirta*: Its chemistry and medicinal uses. Pharmacogn Rev. 2010; 4: 58–61.

[15] Patil SB, Magdum CS. Phytochemical analysis of *Euphorbia hirta* Linn. Int J Pharm Sci Res. 2011; 2: 3098–3101.
[16] Lee MH, Yoon EK, Jung KH, Kim MJ, Kim BH, Lee YJ, et al. Anti-inflammatory activity of plant extracts in macrophages. Molecules. 2010; 15: 3803–3817.
[17] Wirtz S, Neurath MF. Mouse models of inflammatory bowel disease. Adv Drug Deliv Rev. 2007; 59:1073–1084.
[18] Perse M, Cerar A. Dextran sodium sulphate colitis mouse model: Traps and tricks. J Biomed Biotechnol. 2012; 2012: 718617.
[19] Kim JJ, Shajib MS, Manocha MM, Khan WI. Investigating intestinal inflammation in DSS model. J Vis Exp. 2012;(60):e3678.
[20] He Z, Zhou R, Wei X, Chen Y, Zhao Y, Wang Y, et al. Plant extracts reduce DSS-induced inflammation. Biomed Pharmacother. 2019;109:746–753.
[21] Zhang Y, Wang X, Li X, Zhang J, Wang Y, Liu H, et al. NF-κB pathway inhibition in colitis. Int Immunopharmacol. 2018;54:246–253.
[22] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009;30:2785–2791.
[23] Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Phytochemical and antioxidant studies using Folin–Ciocalteu method. J Nepal Chem Soc. 2021; 42(1):45–52.
[24] Pękal A, Pyrzynska K. Evaluation of aluminium chloride colorimetric method for flavonoid determination. Appl Sci. 2020; 10(23): 8408.
[25] Sutha S, Mohan VR, Kumaresan S. GC–MS analysis of bioactive components of *Euphorbia hirta*. J Chem Pharm Res. 2011;3(5):126–130.
[26] Suganthi A, Ravi TK. RP-HPLC estimation of phytochemical markers in *Euphorbia hirta*. Chem Methodol. 2019; 3(1): 39–52.
[27] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199–1200.
[28] Sreejayan N, Rao MNA. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 1997;49(1):105–107.
[29] Cooper HS, Murthy SNS, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest. 1993;69:238–249.
[30] Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology. 1989;96:795–803.
[31] Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105: 121–126.
[32] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973; 179:588–590.
[33] Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta. 1979; 582: 67–78.
[34] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82:70–77.
[35] Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology. 1984; 87: 1344–1350.
[36] Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol. 1982; 78:206–209.
[37] Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2023 update. Nucleic Acids Res. 2023; 51(D1): D1373–D1380.
[38] Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. Swiss Target Prediction: A web server for target prediction of bioactive molecules. Nucleic Acids Res. 2014; 42(W1):W32–W38.
[39] Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards suite: From gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics. 2016; 54:1.30.1–1.30.33.
[40] Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: Protein–protein association networks with increased coverage. Nucleic Acids Res. 2019;47(D1):D607–D613.
[41] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11): 2498–2504.
[42] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009; 4(1): 44–57.
[43] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res. 2000; 28: 235–242.
[44] Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking. J Comput Chem. 2010;31(2):455–461.
[45] Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2(4):152–159.
[46] Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and antioxidant activity. Molecules. 2010; 15(10): 7313–7352.
[47] Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem. 1999;47(10):3954–3962.
[48] Pan MH, Lai CS, Ho CT. Anti-inflammatory activity of natural dietary flavonoids. Food Funct. 2010; 1: 15–31.
[49] Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: Antioxidants and beyond. Am J Clin Nutr. 2005; 81: 215S–217S.